

Bioenergetics and Trophic Impacts of Invasive Indo-Pacific Lionfish

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March, 2010

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Indo-Pacific lionfish, *Pterois volitans* and *Pterois miles*, are non-native marine fish with established populations in the western North Atlantic Ocean and Caribbean Sea. Rapid population growth threatens native fish communities and they are considered invasive species. A bioenergetics model was developed for lionfish and applied to populations inhabiting the western North Atlantic Ocean to model the potential impact of these predators on native reef ecosystems. Model parameters were derived by laboratory evaluation of consumption and respiration rates from 14 to 32° C and fish size ranging from 19 to 400 g. The model was calibrated with laboratory growth and consumption data, and model performance was analyzed to evaluate the parameters most sensitive to error. The optimal temperature for lionfish consumption is 29.8° C. Energy allocated to gamete production reduces female lionfish growth rate compared to males and limits maximum body size. Based on the environmental conditions and observed growth, daily consumption estimates of 393 lionfish · ha⁻¹ could remove up to 2.186 kg prey · d⁻¹ during the summer in the Bahamas. The corroborated model is a useful tool for examining the influence of temperature on predation rates, and exploring the interaction between lionfish and prey.

BIOENERGETICS AND TROPHIC IMPACTS OF INVASIVE INDO-PACIFIC LIONFISH

A Thesis

Presented To

The Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Masters of Science

By

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March, 2010

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ACKNOWLEDGMENTS

Dr. James Morris, a NOAA researcher, conceived of this project and procured funding from the NOAA Aquatic Invasive Species Program to support it. Dr. Morris has shown great faith in me, and I give special thanks to him for the opportunity, guidance, assistance, and friendship he has provided me. I also thank my advisor, Dr. Anthony Overton whose positivity and dedication has led me through this experience. I thank committee member Dr. Jim Rice, who contributed greatly to my understanding of bioenergetics and whose input was cornerstone to my success; and thanks to Drs. Roger Rulifson and Joseph Luczkovich whose classroom mentoring and sage advice will never be forgotten. I thank Kenneth Riley, who contributed to the development of this project, assisted with experiments and critiqued earlier versions of the manuscript.

I am grateful to the many divers from the Center for Coastal Fisheries and Habitat Research who participated in the collection of lionfish used in this project: Brian Degan, Brad Teer, John Hackney, Christine Addison, Ken Brennan, Roger Mays, Roldan Muñoz, David Johnson, Mike Burton, John Burke, and Don Field. Assistance with laboratory experiments was provided by Benjamin Edwards, Wood Oglesby, and John Mohan and appreciated greatly. Thank you to Dr. David Evans, Dr. Jeff Buckel, and Jim Morley for training and use of laboratory equipment. Drs. Craig Harms, DVM and Eric Anderson, DVM provided invaluable assistance with fish health diagnostics. Thank you to Riley Morris who kept my prey tanks full.

I would like to thank my parents, Bill and Marie, for never-ending support and guidance. Biggest thanks go to my wife Shayna, who has followed me up and down the East Coast so that I could build my career and has provided me with love and compassion throughout this journey. The devotion she shows to our son is truly remarkable.

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CHAPTER 1: INTRODUCTION

Invasive Species

An invasive species is a species that is both non-native to a habitat and is likely to cause economic or environmental harm or harm to human health (U.S. Invasive Species Executive Order 13112). For thousands of years, since humans began traveling long distances, intentional and unintentional transplantations of plants, insects and animals have altered ecosystems, shifting them to a new steady state. In recent history, the global human population has grown and become more connected, and the incidence of invasions has increased drastically (Lin *et al.*, 2007).

Freshwater and terrestrial invasions are numerous and costly (Pimentel *et al.*, 2000). Over 400 marine invasive species have been documented in U.S. waters in recent decades (Ruiz-Carus *et al.*, 2006). Many non-native introductions are executed by natural resource managers intending to benefit a system by controlling a native pest (Simberloff & Stiling, 1996) or for fishery development (Ogutu-Ohwayo, 1990). Unintended ecosystem-altering cascading effects can ensue, such as habitat degradation (Dermott & Kerec, 1997), decrease in species diversity and richness (Kaufman, 1992), and changes in community structure and food web dynamics (Jude & Leach, 1999).

Lionfish Description

Lionfish *Pterois spp.*, are very recognizable, their ornate fins and striking coloration make them a prized aquarium specimen. Body color varies, typically orange to red, but sometimes almost black and is covered in multiple thin white bars with irregular patterns. The pectoral fins are large and frilly and can be spread and used to corral and corner prey, or flush prey from the

substrate (Fishelson, 1975). The armored head features knobs and spikes, cheek spines, frilly cirri at the mouth corners, and some individuals have supraocular tentacles (Fishelson, 2006; Morris & Freshwater, 2008). The mouth of a lionfish has many small teeth and opens rapidly, to create suction, then closes, expelling water through the gills to capture whole prey.

All lionfish spines (13 dorsal, three anal, and one pelvic) are venomous. *Pterois* spp. dorsal spines are elongate and slender with anterolateral glandular grooves that run along both sides of the spines. The venom-producing glandular epithelium lies within these grooves. The spine and glands are covered by a thin integumentary sheath. When the spine penetrates flesh, the integumentary sheath is displaced, and the venom is exposed and introduced into the victim (Halstead *et al.*, 1955).

Lionfish are physoclistous and have striated muscles along the dorsal surface of the swim bladder. Many fish species use similar muscles for sound production, and this has been reported for some Scorpaenids, *Notesthes robusta* (Pusey *et al.*, 2004) and *Sebastiscus marmoratus* (Miyagawa & Takemura, 1986), but not for lionfish. Hornstra *et al.* (2004) described how the contraction of these muscles changes the shape and position of the gas bladder and adjusts the fish's orientation or "pitch and yaw" without the use of its fins. Lionfish are often observed inverted along the underside of a rocky outcropping.

Lionfish Taxonomy

Two species of tropical marine Indo-Pacific scorpionfish, the Red lionfish *Pterois volitans* and Devil firefish *P. miles*, are established invasive species in the Northwestern Atlantic Ocean and Caribbean Sea. Lionfishes, including the genus *Pterois*, are Actinopterygian teleosts in the order Scorpaeniformes, the family Scorpaenidae and subfamily Pteroinae. *P. miles* is native to

the Red Sea, Persian Gulf, and Indian Ocean (excluding western Australia) and *P. volitans* is native to the western and central Pacific Ocean and western Australia (Schultz, 1986). Genetic analysis of the two species has identified differences in the mitochondrial DNA (Kochzius *et al.*, 2003) and has supported the distinction of separate species (Hamner *et al.*, 2007; Freshwater *et al.*, 2009a). Meristic characteristics are distinct where the species are geographically separate with *P. volitans* possessing one more dorsal and anal soft fin-ray than *P. miles* (Schultz, 1986). In Indonesia, where the species are sympatric, the meristics can overlap (Freshwater *et al.*, 2009a), and few samples from this region have been included in genetic analyses. While there is no published evidence of hybridization between the two species, additional genetic analysis of specimens from western Indonesia is warranted.

In the Atlantic, the meristic counts can overlap, making genetic analysis the only reliable method of distinguishing species (Hamner *et al.*, 2007). *P. volitans* comprised 93% of lionfish samples from the invaded range analyzed by Hamner *et al.* (2007), and a strong population founder effect was detected. Freshwater *et al.* (2009b) analyzed lionfish mitochondrial DNA from North Carolina and the Bahamas and found that the populations are genetically similar, indicating a common source, but found only *P. volitans* in Bahamian samples. Morris (2009) detected no differences in reproductive morphology between *P. miles* and *P. volitans*. Given that *P. miles* and *P. volitans* are closely related (Hamner *et al.*, 2007; Freshwater *et al.*, 2009a), and likely have evolved as sympatric species (distinguishable as separate species only by genetics in part of range), it is assumed that there is no difference in physiology between the two species. In this study, genetic differentiation was not attempted and both species may be included, *P. miles* and *P. volitans* will be referred to collectively as lionfish.

The Lionfish Introduction

The most likely pathway of the lionfish introduction was the accidental or intentional release of captive aquarium specimens (Semmens *et al.*, 2004). Lionfishes are popular among aquarists. Fast growth and relatively large adult body size cause them to outgrow smaller aquaria, possibly triggering intentional releases. Unintentional releases have also been documented (Courtenay, 1995). Invasion chronology (Schofield, 2009) and genetic analysis (Freshwater *et al.*, 2009b) support the theory indicating South Florida as the potential introduction source, a documented “hot-spot” of marine ornamental introductions (Semmens *et al.*, 2004). Exchange of ballast water is a common vector for many marine invasive species, but is less likely than an aquarium introduction for lionfish (Whitfield *et al.*, 2002; Semmens *et al.*, 2004).

Since 2000, the documented establishment and spread of invasive Indo-Pacific lionfish in the western Atlantic Ocean has gained considerable attention from researchers, media outlets, environmentalists, fishermen, and SCUBA divers. The first documented lionfish sighting in the Atlantic was off the East Coast of Florida in 1985 (Morris & Akins, 2009). Additional sightings were documented from Florida in the 1990s (Courtenay, 1995), and in 2000, confirmed sightings were reported from North Carolina, South Carolina, and Florida. By 2002, lionfish were considered established from Miami to Cape Hatteras, and by 2010, had established populations throughout much of the Caribbean (Schofield, 2009; USGS NAS, 2010). Recently, two lionfish have been confirmed in the southern Gulf of Mexico (Aguilar-Perera & Tuz-Sulub, 2010). Based on thermal tolerance, the range could include the entire Gulf of Mexico, and the eastern South American coast, including the entire coast of Brazil (Kimball *et al.*, 2004; Morris & Whitfield, 2009).

Density estimates of lionfish in their native area range from 2.2 lionfish · ha⁻¹ in Palau (Grubich *et al.*, 2009) to 80 lionfish · ha⁻¹ in the Red Sea (Fishelson, 1997). In the Bahamas, mean densities have been reported as high as 393 lionfish · ha⁻¹ (Green & Côté, 2009). Off the coast of North Carolina, mean densities were reported as 21 lionfish · ha⁻¹ in 2004 (Whitfield *et al.*, 2007) and have increased, with over 400 lionfish · ha⁻¹ observed at some locations in 2007 (Morris & Whitfield, 2009). Invasive species often undergo rapid population growth in a novel environment (Brown, 1989), and the effect that high densities of lionfish will have on community structure and ecosystem function of Atlantic and Caribbean reefs should be measured.

Life History Characteristics

Lionfish in the Indo-Pacific primarily occupy reef habitats to depths of 50 m (Schultz, 1986). In North Carolina, most observations have occurred in 30-100 m depths, as inshore distribution is limited by cold winter water temperature (Kimball *et al.*, 2004). In the warmer waters of the Bahamas and Caribbean, lionfish are common on shallow coastal reefs, inshore waters, and mangrove habitats (Morris & Akins, 2009; Schofield, 2009; Barbour *et al.*, 2010).

Lionfish feed on a variety of fishes and invertebrates, and small fish are the dominant prey item (Fishelson, 1975 and 1997; Morris & Akins, 2009). The diet composition is variable and correlated with the local species community. Lionfish are ambush predators, and Morris and Akins (2009) found as many as 21 fish in the stomach of an individual lionfish. Fishelson (1997) observed the stomach of large adults can expand 30 times in volume to accommodate large meals and that lionfish can withstand starvation for 12 weeks.

Adult lionfish have no known predators and juvenile lionfish may have low vulnerability to predation. Juvenile lionfish have been documented in the stomachs of Bahamian groupers (Maljkovic *et al.*, 2008) and cannibalism by adult lionfish has been observed in captivity (Fishelson, 1997); however, avoidance of small lionfish as a prey item was exhibited in experimental interactions with native Atlantic Serranids (Morris, 2009). Lionfish do not exhibit escape behavior in the presence of potential predators (Morris, 2009). Long dorsal spines and pectoral fins increase the apparent size of juveniles and venom delivery mechanisms in the dorsal, anal and pelvic spines may deter predators.

About 50% of lionfish males reach maturity at 100 mm TL and females at 175 mm TL (Morris, 2009). Spawning frequency analysis by Morris (2009) estimated that female lionfish spawn every 3.6 d from North Carolina samples and every 4.1 d from Bahamian samples (a total annual fecundity of approximately 2 million eggs). The courtship behavior of lionfish is similar to *Dendrochirus brachypterus* and can last for several hours prior to a synchronized ascent towards the surface with release and fertilization of two gelatinous egg masses (Fishelson, 1975). The eggs are buoyant and encompassed within a gelatinous, largely protein matrix. The closely related *Dendrochirus zebra* has similar reproductive features, and Moyer & Zaiser (1981) observed that these egg masses may be chemically defended against predation. After hatching, it is estimated that the time to settlement is approximately 26 d (Ahrenholz & Morris, in press).

Potential Impacts

Invasive lionfish occur at high densities, have few predators, and consume abundant prey. The addition of lionfish to fish communities in the Western Atlantic, Caribbean Sea, and Gulf of Mexico could have profound impacts on the ecology and socio-economics of these important natural resources (Morris & Whitfield, 2009). The reef communities of the Southeast U.S. and Caribbean may be particularly vulnerable to an invasive predator because of pre-existing

ecological stressors. Global climate change is causing a shift in reef communities off the Southeastern U.S. to more tropical species (Quattrini *et al.*, 2004). Coral bleaching has been recognized as a widespread problem in this region, a trend which may worsen with increased temperatures, pollution, and ocean acidification (Hoegh-Guldberg *et al.*, 2007; Hughes *et al.*, 2003). Fish community structures have been altered by systematic removal of top level predators by fishing (Jennings & Polunin, 1996), and most of the economically important reef fishes are currently considered overfished (Jackson *et al.*, 2001). The absence of predatory reef fishes could represent a vacant niche that lionfish may fill. In the eastern Mediterranean, where numerous invasive fish have established populations, niche takeover has been demonstrated for multiple species (Goren & Galil, 2001), and the food web has been altered (Goren & Galil, 2005).

Bioenergetics

Bioenergetics models are becoming increasingly popular in fisheries research. The foundation of fish energetics lies in the first law of thermodynamics which states that all energy must be conserved; it can be transformed, but not created or destroyed. In this perspective, all biological organisms can be viewed as packets of energy. By “counting calories” and tracking the fate of the energy uptake by a fish, a mathematical model can be created and later applied in an ecological framework. Winberg (1960) presented the balanced energy equation which is the basis for these models. All energy consumed as food will be converted to body growth or reproductive tissue, expelled as waste products or used as energy for respiration, digestion, swimming or other activity. Further development of modeling strategies began with Kitchell *et al.* (1974; 1977). Modern models use computer programs, such as Fish Bioenergetics 3.0 (Hanson *et al.*, 1997), the “Wisconsin model” developed at the University of Wisconsin and

distributed by Wisconsin Sea Grant. Ecopath, network modeling software for ecosystems, has bioenergetic equations as an underlying model for each species in an ecosystem.

Models can be used to predict any of the bioenergetic equation components, and the input parameters can be derived from laboratory experiments, field observations, literature, or may be “borrowed” from similar species. Optimal design of a specific laboratory experiment is highly dependent on the intended application of the model. Fish are poikilotherms, and water temperature and body size strongly affect metabolic function. Mathematical equations that accurately reflect the relationship between these factors and consumption and respiration rates are the backbone of bioenergetics models. Therefore, it is important to include measurements at a broad range of temperatures and fish sizes (Hartman & Hayward, 2007). Bioenergetics models are more commonly used to predict consumption rather than growth (Hartman & Hayward, 2007).

Bioenergetics have been used to model all types and life stages of fishes from eggs and larvae (Madon & Culver, 1993) to small, freshwater species including mosquitofish, *Gambusia holbrooki* (Chipps & Wahl, 2004) to large-bodied tunas, *Thunnus spp.* (Boggs, 1984) as well as invertebrates (Schneider, 1992). Questions addressed with bioenergetics models include, but are not limited to: larval fish consumption rates (Worischka & Mehner, 1998), habitat suitability (Niklitschek, 2001), predator-prey interactions (Wahl & Stein, 1988), consumption by fish populations (Hartman & Brandt, 1995), optimizing aquaculture conditions (Claireaux & Lagardere, 1999), and measuring mercury bioaccumulation (Korhonen *et al.*, 1995).

In this study, a bioenergetics model was created to predict food consumption by lionfish. It is designed to be used as a tool for measuring impacts on fish communities in the current and future invaded range. Controlled laboratory experiments were conducted to determine the effect

of temperature and body size on the consumption and respiration rates of lionfish across the range of thermal tolerance. The energy content of lionfish, lionfish egg masses, and experimental prey were measured using proximate chemical composition analysis. Independent growth trials were conducted to calibrate the model, and parameter sensitivity was tested and found to be low. The model was then applied to a population of lionfish from data collected in the Bahamas in 2008.

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CHAPTER 2:

DEVELOPMENT AND APPLICATION OF A LIONFISH BIOENERGETICS MODEL

Introduction

The Indo-Pacific Red lionfish, *Pterois volitans* (Linnaeus, 1758) and Devil firefish, *Pterois miles* (Bennett, 1828) represent the first successful invasion of non-native marine carnivorous fishes in the western North Atlantic (Meister *et al.*, 2005). Likely introduced through the aquarium trade (Courtenay, 1995; Whitfield *et al.*, 2002), lionfish are established and becoming increasingly abundant in hard-bottom and reef habitats off the Southeast United States, Bermuda, the Bahamas, and the Caribbean (Schofield, 2009). They have also been documented in the southern Gulf of Mexico (Aguilar-Perera & Tuz-Sulub, 2010). Lionfish commonly inhabit reef habitats to depths of 50-100 m (Schultz, 1986; Whitfield *et al.*, 2007). On the U.S. East Coast north of Florida lionfish are not common in near-shore waters because of winter temperature limitations (Kimball *et al.*, 2004), but are common on shallow coastal reefs, and in inshore waters and mangrove habitats in the warmer waters of the Bahamas and Caribbean (Morris & Akins, 2009; Schofield, 2009; Barbour *et al.*, 2010).

Both *P. volitans* and *P. miles*, are sympatric species of the family Scorpaenidae, subfamily Pteroinae. They have been identified in the Atlantic through genetic analysis, though *P. volitans* represented 93% of samples (Hamner *et al.*, 2007). However, a recent study did not detect *P. miles* in samples collected in the Bahamas (Freshwater *et al.*, 2009a). *P. miles* is native to the Indian Ocean and the Red Sea and has invaded the Mediterranean Sea via the Suez Canal (Golani & Sonin, 1992). *P. volitans* occurs mostly in the Pacific, its range overlaps with *P. miles* in western Indonesia. Initial mitochondrial DNA analysis did not distinguish if they were

separate species or two populations of the same species (Kochzius *et al.*, 2003). Hamner *et al.* (2007) and Freshwater *et al.* (2009b) confirmed the separation of species. In the Atlantic Ocean, morphometric and meristic characteristics can overlap, making it difficult to distinguish between the two species (Hamner *et al.*, 2007). To date, no reproductive differences have been observed between *P. miles* and *P. volitans* (Morris, 2009). For this study, both species are collectively referred to as lionfish, and it is assumed that there is no difference in physiology between the two species.

Lionfish are generalist piscivores; most fish species common to the habitat are represented in the diet including juveniles of the economically important families Serranidae (groupers) and Lutjanidae (snappers) (Morris & Akins, 2009). Finfish represent >78% of lionfish diet by volume in the Bahamas (Morris & Akins, 2009). Finfish become more important through ontogeny where adults >260 mm TL are almost exclusively piscivorous (>90% by volume) (Morris & Akins, 2009). Lionfish can consume prey up to 48% of its body length (Morris & Akins, 2009). Lionfish predation reduced recruitment of forage fishes to experimental patch reefs by 79% (Albins & Hixon, 2008). Direct predation by lionfish may inhibit the recovery of suppressed stocks of the snapper/grouper complex in the Atlantic Ocean, and adults of these species might also compete with lionfish for space and food (Morris & Whitfield, 2009).

Lionfish populations may have a considerable impact on reef communities (Meister *et al.*, 2005) by altering food web structure and species interactions. Therefore, there is an increasing need to determine the trophic impacts of the lionfish invasion. Fundamental to understanding and predicting lionfish trophic impacts is the quantitative assessment of lionfish energetic requirements. Here, a bioenergetics model was constructed to predict consumption rates of lionfish across various temperatures of the invaded range.

The specific objectives of this study were to: 1) develop parameters for the bioenergetics model through a series of laboratory experiments, 2) calibrate the model via independent growth trials, 3) conduct sensitivity analysis of each model parameter, 4) use the model to estimate field consumption rates as a proportion of the maximum, 5) demonstrate differential growth between male and female lionfish, and 6) estimate total consumption rate of lionfish populations.

Bioenergetics Model Development

Materials and Methods

Model Overview

The bioenergetics of lionfish is summarized by the balanced energy equation: $C = G + R + S + F + U$, where C is the total energy consumed, which is partitioned into somatic and gonadal growth G , metabolism as respiration R and specific dynamic action $S = SDA \cdot (C - F)$, and waste products as egestion $F = FA \cdot C$ and excretion $U = UA \cdot C$ (Winberg, 1960). Somatic growth estimates are typically derived from field measurements, and gonadal growth is lost during spawning events. C and R are described by temperature and weight-dependent functions, while SDA , FA , and UA are constants. The consumption model follows the equation $C = C_{max} \cdot p \cdot f(T)$ (Hanson *et al.*, 1997), where C is the energetic equivalent of specific consumption measured in $\text{g prey} \cdot \text{g lionfish mass}^{-1} \cdot \text{d}^{-1}$, $C_{max} = CA \cdot W^{CB}$ is a weight-dependent function of the maximum rate, p is the proportion of the physiological maximum consumed and $f(T)$ is a temperature dependence function. The respiration model follows the equation $R = RA \cdot W^{RB} \cdot f(T) \cdot ACT$, where R is the energetic equivalent of the weight-specific respiration rate measured in $\text{g O}_2 \cdot \text{g lionfish mass}^{-1} \cdot \text{d}^{-1}$; RA is the intercept of the weight-dependent function and RB is the

slope; $f(T)$ is the temperature-dependence function and ACT is the activity multiplier. Most parameters for the lionfish model were derived from laboratory experiments (Table 1).

Laboratory Experiments

Fish Acquisition

Lionfish (n=70) were collected by SCUBA divers in 2007 and 2008 approximately 35 km south of Beaufort Inlet, North Carolina, USA. They were transported live to holding tanks and aquaria at the Center for Coastal Fisheries and Habitat Research in Beaufort, North Carolina. Lionfish <30 g were rare in the wild collections; therefore an additional 30 lionfish (1-10 g) were obtained from the Phillipines via an aquarium supplier. All lionfish were acclimated to the laboratory for approximately one month prior to experimentation.

Consumption

To investigate the relationships between maximum consumption (C_{max}) of lionfish, temperature, and fish size, C_{max} was measured in the laboratory at seven temperatures from 14.4-32.5° C, with lionfish ranging in size from 19-400 g. Kimball *et al.* (2004) observed cessation of feeding at 13° C, so 14° C was set as the target temperature for the lowest treatment; 32.5° C proved to be the maximum temperature achievable without detrimental stress to the fish (personal observation). Consumption was measured for individual lionfish in 20, 40, or 110 l glass aquaria placed in a water bath and connected to a recirculating seawater system with biological and mechanical filtration and temperature controlled by a heat pump. System water flowed through each aquarium with a minimum 10 daily turnovers. Fish were acclimated to the experimental temperature at a maximum of 1° C · d⁻¹ then fed *ad libitum* for a minimum of 5 d prior to initiating a feeding trial. Live mummichogs, *Fundulus heteroclitus* were selected as prey because they are a hardy species, survive well in captivity, and were readily available.

Mummichogs were held in tanks with flow through sea water at ambient temperatures and fed *ad libitum* once daily with a marine fish crumbled diet. Prey fish ranged from 0.2 to 4.0 g and individual TL was <40% lionfish TL. The mass and number of prey was recorded upon addition to lionfish tanks. Tanks were visually inspected daily to estimate the number of prey consumed. Consumed prey was replaced with live prey; prey that died but were not consumed, and prey remaining at the end of the trial, were removed and the unconsumed mass subtracted from the total mass of prey added to determine total consumption during the trial. Presence of live prey remaining in the tank at all times after initial feeding indicated that fish were feeding *ad libitum* (Whitledge *et al.*, 2003). Trials generally lasted 6-9 d. During the 14 °C trial few feeding events were observed during the initial period, so it was extended to 15 d to allow for enough feeding events to accurately estimate mean daily consumption rate. Some fish were tested at multiple temperatures, because of the limited availability of lionfish and the growth that occurred during the study period. No individuals were used in consecutive trials. Temperature was recorded hourly by a digital temperature logger, salinity was maintained between 32 and 36 ppt, and ammonia-nitrogen and nitrite-nitrogen concentrations remained < 1 mg · l⁻¹.

Respiration

Resting metabolic respiration rates of lionfish (n=25) from 19 to 309 g were measured at three temperatures (mean ± SD): 17.5 ± 0.4° C, 23.3 ± 0.9° C, and 29.1 ± 0.4° C. Fish were placed in automated intermittent flow respirometers, with chamber sizes modified to suit fish size. Small fish (≤100 g) were placed in a gasket-sealed 4.3 l acrylic tube and larger fish in a 40 l glass aquarium with a sealed lid. Systems were submerged in a temperature controlled water bath. Water was pumped into respirometers from an aerated reservoir containing seawater that had passed through a 5 µm paper cartridge filter and ultra-violet sterilizer to minimize microbial

respiration. The recirculating pump ran continuously and the flush pump ran intermittently (timer controlled) to supply the system with oxygenated water. Laminar flow through the chambers at $0.1 - 0.3 \text{ cm} \cdot \text{s}^{-1}$ allowed the fish to be in a resting state. An automated data logger attached to a galvanic dissolved oxygen (DO) probe recorded DO concentrations at 5-60 second intervals throughout the trial. Between flushes, the system was closed and the observed rate of decline in DO was used to calculate specific rate of respiration in $\text{g O}_2 \cdot \text{g fish}^{-1} \cdot \text{d}^{-1}$. The length of interval between flushes was adjusted to maintain minimum DO levels above $5 \text{ mg} \cdot \text{l}^{-1}$. After the fish was acclimated to the chamber, a minimum of five cycles was recorded for each fish, and the mean rate used for further analyses. At least two trials at each temperature were run with no fish in the chamber to account for microbial consumption of oxygen, and none was detectable.

Excretion, Egestion, and SDA

Total ammonia nitrogen (TAN) excreted into the water at 27°C was measured for nine individual lionfish 4-15 g to estimate excretion rate. Fish were placed in 10 l containers with flow-through ammonia-free seawater. Each fish was fed grass shrimp (*Palaemonetes spp.*) to satiation (2-5 individual prey) and the mass consumed recorded. The TAN concentration was measured hourly for 12 h by which time the concentration was zero. The TAN concentration for each hour interval was multiplied by the flow rate and the resulting mass was then summed for the entire period. The mass of ammonia-nitrogen was converted to energy using the oxycaloric coefficient of $0.0249 \text{ KJ} \cdot \text{mg}^{-1}$ (Elliot & Davidson, 1975). The energy density of grass shrimp was reported by Anderson (1974) and de la Cruz (1983) as $18.42 \text{ KJ} \cdot \text{g}^{-1}$ dry weight; the wet weights were converted to dry weights using the formula $x = 0.254y + 0.05$, where x is individual dry weight and y is individual wet weight (Anderson, 1974). The amount of energy excreted as ammonia-nitrogen was divided by the amount of energy consumed to predict UA .

The proportion of energy egested (*FA*) was calculated based on fecal production reported by Fishelson (1997). He reported the range of masses of dried feces produced by lionfish fed known meal sizes (40-80 g of live tilapia *Oreochromis spp.* yielded 1.03-3.03 g dry feces and 20-24 g of mosquitofish *Gambusia affinis* yielded 0.92-1.45 g dry feces). The prey fish wet weights were converted to dry weights using the formula $D = 0.24W$, where D is whole fish dry weight and W is whole fish wet weight. This equation is based on the reported moisture content for tilapia (Wang *et al.*, 2002; Tartiel *et al.*, 2008) and closely matches the moisture content of mummichogs measured in this study. The proportion of mass remaining as feces was calculated for the minimum and maximum consumption reported by Fishelson (1997) and the mean of those values is reported as *FA*. *SDA*, the metabolic cost of digestion, is an insensitive parameter typically between 0.15 and 0.2 (Hanson *et al.*, 1997). High protein diets of carnivores require more digestive energy (Tytler & Calow, 1985), so a value of 0.2 was chosen for the lionfish model.

Energy Density

Gross energy content was measured by proximate chemical composition analysis on samples of whole lionfish, released egg masses collected in the laboratory, and whole mummichogs. Samples were freeze-dried, homogenized, and a subsample was weighed and lipids extracted in a Soxhlet apparatus with petroleum ether as the solvent (Dobush *et al.*, 1985). Ash content was determined by burning off all remaining organic content at 450° C for 12 hr in a muffle furnace. Carbohydrate content of fish is assumed to be zero (Henken *et al.*, 1986). The protein content was calculated by subtracting the percent fat and ash from 100%. Proportional dry matter compositions were multiplied by caloric values of 0.0396 and 0.0237 KJ · g⁻¹ for fat

and protein, respectively (Henken *et al.*, 1986) and summed. This methodology gives results equivalent to combustion in a bomb calorimeter (Henken *et al.*, 1986).

Model Construction

Model parameters for C were determined from the temperature and weight-dependent functions derived from experimental data. Weight-specific mean daily consumption rate at the optimal temperature was plotted against fish mass, and an allometric mass function in the form $C_{max} = CA \cdot W^{CB}$ was fit to the data using a least squares regression, where CA is the intercept of the function, CB is the slope, and W is the fish wet weight. For determination of temperature dependence ($f(T)$), equation 2 from Kitchell *et al.* (1977) for temperature dependence in warm water fishes was used to determine the optimal temperature using an optimizer, Microsoft Excel® Solver (Redmond, WA). $f(T) = V^x \cdot e^{(x(1-v))}$, $V = (CTM-T)/(CTM-CTO)$, $X = (Z^2 \cdot (1+((1+40/Y)^{0.5})^2)/400$, $Z = \ln(CQ) \cdot (CTM-CTO)$, $Y = \ln(CQ) \cdot (CTM-CTO+2)$ where CTO is the optimal temperature at which maximum consumption occurs, CTM is the lethal thermal maximum, and CQ approximates a Q_{10} over relatively low water temperatures. CTM was set at 34.5° C because mortality was observed at that temperature during an attempted consumption trial; this value is assumed to be near the thermal maximum for lionfish and is within the range of lethal maxima for tropical marine fishes (Menasveta, 1981). Values for CA and CB were entered into the consumption equation along with the experimental data for W , T and C . The optimizer calculated the values for CQ and CTO that minimized the sum of squares of the residual values between calculated and observed consumption rates.

The weight and temperature-dependence of respiration was modeled using the same function (Equation 2), but with different parameters. Parameters for the respiration model were

derived from experimental respirometry data. The slope (RA) and intercept (RB) of the allometric mass function were determined from the negative power regression of data from the 29.1° C trial. Based on consumption trial results, this temperature is near the lionfish metabolic optimum. Resting metabolic rate as a function of temperature was fit using equation two (Hanson *et al.*, 1997). RTM , the thermal maximum, remained 34.5° C. RTO , the optimal temperature was fit to the function and set at 32° C. RQ was calculated with the same method as CQ using respiration rates of lionfish > 19 g at 17.5 and 23.3° C.

Model Calibration and Determination of ACT

Thirteen individual lionfish 121-693 g were held in 100 l tanks and fed various rations (0.10-0.35 g · g⁻¹ · d⁻¹) of live prey for 19-20 d. Each individual was weighed at the beginning and end of the growth trial, and the mass of prey consumed determined. Water temperatures were recorded daily. Mean temperatures for the trials ranged from 21° to 28° C. The results of these trials were used to calibrate the model by entering growth and daily temperature values, and comparing predicted consumption to observed consumption. The model was calibrated by iteratively adjusting the activity multiplier ACT to minimize the percent error of model predictions during validation trials where both feeding and growth were known. The resulting value for ACT was 1.8, which is within the range of commonly used values (Tytler & Calow, 1985).

Sensitivity Analysis

A sensitivity analysis was conducted to identify the relative influence of parameters on model output. One individual growth trial was selected, representing a median lionfish size and

feeding ration. The model was run repeatedly, and each parameter was individually altered $\pm 10\%$ in each direction, except temperatures were adjusted $\pm 1^\circ \text{C}$, and the predicted mass of prey consumed was recorded (Kitchell *et al.*, 1977). The mean percent change in consumption was calculated for each parameter.

Results

Laboratory Experiments and Model Construction

Consumption

Results from 75 individual C_{\max} trials were used to fit the weight-dependent and temperature-dependent functions for consumption. The best-fit weight-dependent function is $C = 0.603W^{-0.465}$ at 29.7°C ($N = 10$, $R^2 = 0.92$) (Fig. 1). Within $f(T)$, the Q_{10} (CQ) is 4.0 based on linear regression ($N = 16$, $R^2 = 0.74$). When the experimental data was fit to Equation 2, the optimizer predicted the optimal temperature for consumption (CTO) to be 29.8°C (Fig. 2).

Respiration

Results from 36 individual respiration trials were used to fit the weight-dependent and temperature-dependent functions for respiration. The best-fit weight-dependent function is $R = 0.0085W^{-0.28}$ at 29.1°C ($N = 11$, $R^2 = 0.42$) (Fig. 3). Within $f(T)$, the calculated Q_{10} (RQ) is 2.08 ($N = 11$, regression $R^2 = 0.70$).

Egestion and Excretion

The proportion of consumed energy expelled as waste products was 0.26. The calculated energy content egested as feces from Fishelson (1997) was 20% of the consumed prey ($F = 0.20$). Results from the excretion trials determined that 6% of the energy in consumed prey was

excreted as aqueous ammonia-nitrogen ($U = 0.06 \pm 0.04$ SD). These values are similar to those used in bioenergetics models of other carnivorous fishes (Hanson *et al.*, 1997).

Energy Density

The energy density of lionfish increases with body size (Fig. 4). Lionfish energy density (ED in $\text{KJ} \cdot \text{g}^{-1}$) can be inferred from percent dry weight using the formula $ED = 0.331d - 3.548$ ($R^2 = 0.91$) (Fig. 4a), where E is total energy (KJ) and d is the percent of whole lionfish wet weight remaining after drying. In terms of wet weight, energy density of lionfish exhibits an allometric increase and can be described by $ED = 2708w^{0.1504}$ ($R^2 = 0.85$), where w is the fish wet weight (Fig. 4b). The total energy content of an average spawn for a female lionfish is estimated to be 31.3 (± 17.3 SD) KJ, based on the proximate analysis of five egg masses collected from a holding tank immediately after being released by three females within 24hr after capture from the wild.

Model Calibration

The predicted consumption for specific growth intervals observed during 13 19-20 d laboratory growth trials was compared to the measured mass of prey consumed during each trial (Fig. 5). Eight of the model predictions had an error $\leq 15.0\%$ and the remaining observations were within 40% of the consumption estimate for observed temperature and growth during the trial. The mean error was $-5.4\% \pm 2.1$ SD.

Sensitivity Analysis

Overall, the model exhibited very low sensitivity to 10% perturbations in individual parameter estimates. A 10% reduction in prey energy density is the only alteration that resulted in greater than 10% change in predicted g of prey consumed (+11.6%). The Respiration model

parameters RB, RA, and ACT had the next highest sensitivity rankings, with 4% to 6% impact. All of the consumption parameters had low sensitivity, with the result changing less than 1% (Fig. 6).

Model Application

Methods

Consumption Rate

The rate of lionfish consumption as a proportion of C_{\max} (p) was determined by entering field estimates of growth and temperature into the Fish Bioenergetics 3.0 lionfish model, and iteratively solving for the p value. Growth was directly measured in a mark-recapture study, and calculated from a preliminary size-at-age analysis from otolith annuli. The length-weight regression equation $W = .000002285L^{3.335}$ ($R^2 = 0.97$) from pooled collections of 782 individuals from North Carolina and the Bahamas (J. Morris, National Oceanic and Atmospheric Association (NOAA), unpublished data) was used to convert total lengths to wet weights.

In-situ growth rates of eight individual lionfish were measured in the field in 2007-2008. The lionfish were tagged using Streamer tags (Floy Tag Seattle, WA) and recaptured in the Bahamas after being at-large for 29-178 d (mean = 64 d); total length was measured at each capture (L. Akins, Reef Environmental and Education Foundation, S. Green, Simon Frasier University & J. Morris, NOAA, unpublished data). Sea surface temperatures (SST) for this area were obtained for each at-large period from the NOMADS database (Rutledge *et al.*, 2006). SST is representative of bottom temperatures in water depth less than 30m (Kimball *et al.*, 2004). The mean of the proportional consumption rates (p) calculated by the model for these individuals is 0.6 ± 0.15 SD.

An estimate of annual growth was obtained using a Von-Bertalanffy growth function developed from otolith analysis of 134 individuals from North Carolina (J. Potts, National Marine Fisheries Service, unpublished data). While the annual increments have not yet been validated for this species, the curve provides an estimate of annual growth by the population. Each year's growth up to age eight was entered into the model with mean sea surface temperatures for each month from offshore North Carolina (NOMADS, Rutledge *et al.*, 2006). The mean p -value from the eight year-long simulations was 0.6 ± 0.06 SD.

Because of the strong agreement between the short-term and long-term estimates of proportional feeding rate, $p = 0.6$ was considered to be a reasonable value and was applied in all further modeling scenarios. All other model parameters reported in Table 1 were also held constant: only lionfish size, energy density (as a function of size), and temperature were adjusted.

Sex-Specific Growth

To demonstrate the effect that lionfish reproductive output has on growth, a male and female lionfish were modeled feeding at the same proportion of C_{\max} ($p = 0.6$) for one year, only the female spawning was different between the two. The simulation began on January 1, 2008 and used mean monthly sea surface temperatures from the mid-Florida Keys (24.6° - 29.7° C) (NOMADS; Rutledge *et al.*, 2006). Starting size was set at 100 g (about 200 mm TL) because all males and >90% of females this size are sexually mature (Morris, 2009). Spawning frequency analysis reported by Morris (2009) estimated that female lionfish spawn on average every 3.85 d; thus, one spawn every 4 d was used in this exercise. Dry weight and energy content of five recovered egg clutches was measured after females ($n=3$) each released 2 gelatinous egg masses in the laboratory one day after collection from the wild. One female was captured and euthanized

immediately after spawning and freeze-dried along with her eggs. The energy content of the eggs equaled 1% of the energy content of the female; so 1% of the female's energy was lost every four days. Male spawning energetic output was considered negligible. Male gonads average 0.1% of the fish mass by wet weight and approximately 0.05% of total fish energy content according to proximate chemical composition analysis of the testes of eight male lionfish. Since only a fraction of the testes can be released as sperm each day, the loss is unlikely to significantly hinder growth.

Consumption by Lionfish Populations

The size structure of lionfish population was defined by length-frequency analysis of 586 lionfish collected in the Bahamas in 2008 (L. Akins, Reef Environmental and Education Foundation, & J. Morris, NOAA, unpublished data). The fish were collected by SCUBA divers that attempted to collect every lionfish as it was encountered; thus, size bias is considered minimal. The population was divided into four size classes and the median size fish in each size class (SC) was converted to mass via the length weight regression equation, (SC1 = 8.8 g (6.1%), SC2 = 82.3 g (35.7%), SC3 = 309.6 g (49.7%), SC4 = 834.1 g (8.5%)) and was modeled to calculate daily consumption at ten temperatures, from 14° to 32° C, at 2° intervals, at a range of lionfish densities. The consumption for each size class was multiplied by the proportion of the population represented and the number of lionfish in the population to yield daily population consumption rates at each temperature.

Consumption rates were calculated for a lionfish population with the density of 393 fish · ha⁻¹ reported by Green & Côté (2009) off southwest New Providence Bahamas. Monthly sea surface temperatures were obtained for this location, and daily consumption for a population with the size structure described above was summed to obtain monthly and annual consumption.

The size structure was assumed to be the same each day and a constant proportional feeding rate of 0.6 was applied.

Results

Sex-Specific Growth

During the one year simulation, female lionfish grew from 100 g (~200 mm TL) to 294 g (~270 mm), while males grew to 496 g (~315 mm). Total consumption was 1,830 g by a female and 2,226 g by a male (Fig. 7). Males consumed 21% more prey and gained twice as much mass. Females allocated 20% of the total consumed energy to somatic growth and 12.6% to egg production.

Consumption by Lionfish Populations

The daily consumption estimates of lionfish populations varied greatly with temperature and population density. Consumption estimates increase nearly eight fold from 14° C to 30° C, and increased in direct proportion to population density (Fig. 8). The simulation of a New Province population of 393 lionfish · ha⁻¹ predicts daily consumption of 2.124 - 2.186 kg of prey from June through October when mean temperatures are greater than 28° C. The cumulative annual consumption is 680 kg · ha⁻¹; if the diet is 78% finfish (Morris & Akins, 2009), then 530 kg · ha⁻¹ of fish would be removed by lionfish consumption.

Discussion

Lionfish are capable of consuming the most prey when water temperatures approach 30° C, and lionfish populations at reported densities are estimated to consume over a half tonne of prey fish per year within one hectare of reef habitat. While the replenishment rates of lionfish prey are unknown, it is probable that lionfish populations are causing substantial trophic impacts

on the food web dynamics of these ecosystems. The correlation of consumption rate with temperature indicates that the rate will vary seasonally in the temperate areas of their range, but has the potential to remain high in the tropics throughout the year.

Some studies have evaluated the metabolism of warmwater marine reef fish adults (Kline, 2004; Claireaux & Lagardere, 1999) and juveniles (Weunschel *et al.*, 2004). However, lionfish have several unique characteristics with unknown associated energetic demands that make them difficult to compare with other modeled fish, including venom defense, large pectoral fins, slow swimming speed and skin sloughing (Fishelson, 1973). While many bioenergetics models “borrow” parameters determined for other species with similar body size, feeding habits, activity level, and life history characteristics, it was prudent to develop as many species-specific parameters as possible for lionfish.

The previous study most comparable to this study is an assessment of the impact of invasive roi, *Cephalopholis argus* (Bloch & Schneider, 1801) on Hawaiian reefs estimated by a gastric evacuation model (Dierking, 2007). *C. argus* was intentionally released, along with several other species, for fishery development in the Hawaiian Islands in 1956 (Randall, 1987). Because of concerns of ciguatera poisoning, it is banned from sale in fish markets (Dierking & Campora, 2009) and there are concerns regarding the negative impact these fish are having on the reef communities. Dierking (2007) estimated that the local roi population removed 11.2% of the standing stock biomass of the reef system annually, or an average of 0.03% each day. The *C. argus* study objectives were similar to those presented here, and roi and lionfish occupy similar marine reef habitats. Both estimates, using different techniques, are of the same order of magnitude which indicates the predictions may have merit. The daily estimate of % fish biomass consumed by a Bahamian lionfish population is six-fold higher than roi, indicating that the

impact on the fish community by lionfish may be greater, or that differences among methods exist.

Fish energy budgets change with fish size, and some species have separate model parameters for juveniles and adults. In the population structure applied in this modeling exercise, only 6% of the fish were ≤ 30 g, and consumption by this group was $< 0.2\%$ of the population total, indicating that even if there are differences not captured by the size-dependent variables, the resulting error is insignificant. Development of a separate juvenile model may be useful if coupled with better estimates of juvenile abundance.

Laboratory growth trials were conducted, and the activity multiplier was set at 1.8 to fit the model to those growth data. This value is similar to the *ACT* estimated for southern flounder, *Paralichthys lethostigma* (2.1), another recognized low activity piscivore (Burke & Rice, 2002). Fishelson (1997) reported on the consumption and growth of *P. volitans* [*P. miles*, (see Hamner *et al.*, 2007)], and model simulations using the reported temperature, prey type, lionfish size and growth, gave consumption estimates considerably lower than those reported. Adjusting the activity multiplier to greater than five predicted consumption similar to Fishelson's (1997), but this is unrealistically high for the activity level observed and reported for lionfish in aquaria or in the wild. Additionally, with the elevated *ACT* the model sensitivity increased considerably.

Possible explanations for the differences in growth trial results between the two studies include: 1) varied duration of growth periods (Fishelson's (1997) growth intervals ranged from 1-14 months vs. 19-20 d in this study); 2) geographically and genetically different populations of lionfish used in each study, and subsequent potential differences; 3) different laboratory conditions such as tank size, habitat availability (Fishelson (1997) provided structure, but this study did not), prey type, and feeding schedule changed fish behavior, metabolic function or

both. However, an annual estimate of population consumption of lionfish in the Red Sea using the bioenergetics model, given the same population and environmental assumptions, provides similar results. Fishelson (1997) estimated a population of 80 lionfish of 300-400 g body size to consume $228.48 \text{ kg} \cdot \text{yr}^{-1}$. Modeling the cumulative daily consumption of 80 350 g lionfish at 25.5° C for one year with a p -value of 0.6 using the bioenergetics model estimates $181.04 \text{ kg prey} \cdot \text{yr}^{-1}$ consumed. This is approximately 80% of the consumption predicted by Fishelson (1997), whose laboratory methods stated *ad libitum* feeding (i.e., $p = 1$).

The population estimates of consumption assume that fish are feeding at 60% ($p = 0.6$) of physiological capacity, because this was the mean rate fit to field measurements of growth. There is individual variability in proportional feeding rates, and it is unknown if proportional feeding rates change seasonally or with changes in prey availability. Additional estimates of growth rates should be obtained for both male and female lionfish from both direct measurement (tagging studies) and otolith increment analysis. The samples used to determine growth rates did not include sex, and given that females grow slower because of year round spawning, the consumption rates reported here should be considered conservative.

Harbone *et al.* (2008) reported mean fish biomass densities from surveys of several Bahamian reefs of $742 \text{ kg} \cdot \text{ha}^{-1}$ and the data within Lang (2003) indicate similar fish densities near Andros Island in 1997 and 1998. The daily fish consumption estimates calculated for 393 Bahamian lionfish $\cdot \text{ha}^{-1}$ are 0.17-0.21% of this total fish biomass.

Biological and ecological models inherently incorporate a certain degree of error. The sensitivity analysis used to evaluate the lionfish bioenergetics model is a simple one and does not account for the potential of multiple sources of error or interactions between parameters; however, it does show that the sensitivity of each individual parameter is fairly low. Prey energy

density and respiration model parameters have the greatest sensitivity to error. The respiration parameters are typically sensitive in this type of model (Rice *et al.*, 1983). Prey energy density is not commonly the most sensitive parameter. The low sensitivity of other parameters may be a result of low resting metabolic rates and high consumption rates. The energy density of prey used for laboratory trials was measured and variability was low. The same prey energy density was also used during model simulations of lionfish in reef habitats because estimated energy content for species common to lionfish diet were not available in the published literature. Deriving the majority of parameters from direct laboratory measurement improves the robustness of the model. Bioenergetics models typically most precisely estimate consumption from observed growth (Kitchell *et al.*, 1977), and in some instances consumption rate can be modeled solely as a function of temperature and fish size (Bartell *et al.*, 1986). Strong agreement between the calibrated model's predictions of prey consumption and observed rates in the laboratory trials invoke further confidence that the lionfish model will accurately predict consumption.

Gamete propagation clearly has a major effect on the somatic growth of female lionfish. This is corroborated by the observation that all of the largest lionfish (>390 mm TL) observed in collections (N>2000) thus far, are males (J. Morris, NOAA, unpublished data). Size-at-age analysis of lionfish populations will help to elucidate the sex-specific growth on a population level once a validated Von-Bertalanffy Growth Function is created for each sex. While Morris (2009) confirmed that spawning occurs throughout the calendar year, additional estimates of spawning periodicity are needed to determine if it varies seasonally or with temperature.

The "Wisconsin" Fish Bioenergetics 3.0 model platform was chosen because it has a large distribution (>1000) (Hartman & Kitchell, 2008), and the Microsoft Windows-based format is user friendly. Researchers and managers will be able to model potential or realized impacts of

lionfish on local fish communities. This type of model initially was developed for freshwater fishes in the 1970s and applied mostly to lake systems (Hewett & Johnson, 1992). In the decades since, the diversity of applications has expanded to include invasive species such as sea lamprey *Petromyzon marinus* (Kitchell, 1990) and zebra mussel *Dreissena polymorpha* (Schneider, 1992); and marine fish such as cod *Gadus morhua* (Hansson *et al.*, 1996), tunas *Thunnus albacares*, *Euthynnus affinis*, and *Katsuwonus pelamis*. (Boggs & Kitchell, 1991) and rockfish *Sebastes spp.* (Harvey, 2005). This unique application of the platform to an invasive, tropical marine reef fish expands the breadth of model applications and provides scientists a tool to measure the impact of lionfish in the invaded range, which is presently expanding.

The ecological consequences of lionfish establishment and dispersal in the Atlantic will be realized in the coming decades. This physiological model describes how lionfish predation and growth are affected by temperature, and will be a valuable tool for estimating the consumptive impacts on growth of lionfish populations. Additional data on growth of lionfish in specific habitats or regions will be essential for estimating lionfish feeding rates. These estimates can be obtained readily by mark-recapture studies, otolith increment analysis, or both. Also, measurement of the energy content of common reef fish species will improve model performance, because prey energy density is the most sensitive parameter.

The trophic impacts of lionfish are likely to be the greatest consequence of the invasion. This study demonstrates that lionfish populations at the high densities observed in the Atlantic are capable of consuming a measurable proportion of the standing stock biomass of reef communities. The influence this consumption will have on the community structure and food web dynamics of the system merits further investigation. Measures of replenishment rates or turnover of prey fish biomass within these systems are needed to elucidate the relative scale of

this consumption estimate. Additionally, estimates of in-situ growth of lionfish in different habitats, coupled with estimates of local prey abundance, will allow for accurate estimates of the proportional feeding rate of lionfish populations, which is likely site-specific and may have seasonal trends. In order to measure the impact lionfish consumption on community structure and food web dynamics, lionfish can be incorporated into network models that have been developed for marine reef ecosystems (Opitz, 1993; Deehr *et al.*, 2007). Many studies have characterized reef community structure (e.g. Harbone *et al.*, 2008) and recruitment (e.g. Tolimieri *et al.*, 1998), and observed patterns may change as lionfish alter energy flow within these food webs.

High-quality site-specific data are needed to accurately define simulation inputs, and the habitat variability and geographic scale of the lionfish invasion is immense. Other applications of the model may provide useful relative comparisons. Gag grouper, *Mycteroperca microlepis* (Goode & Bean, 1879) is a valuable game fish and adults occupy reef habitats currently being invaded by lionfish. While a complete gag grouper bioenergetics model has not been published, metabolic rates (Kline, 2004) have been measured, and gastric evacuation models have been developed (Berens, 2005). Development of a bioenergetics model for gag might provide insight to how lionfish energetics and consumption rates compare to native predators, giving more meaning to observed changes in reef fish community structure.

This study represents the first complete bioenergetics model for a tropical marine reef fish developed by laboratory experimentation. The low sensitivity of parameters and strong agreement of model predictions with laboratory growth data indicate this model accurately reflects the physiological characteristics of invasive lionfish. Future research can use the

framework provided here and further calibrate the model while expanding its capabilities and applications to more fully quantify the impacts of lionfish on reef communities and fisheries.

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Table 1. Parameters of Fish Bioenergetics 3.0 (Hanson *et al.*, 1997) model for lionfish.

Parameters were derived from data collected in laboratory experiments in this study except

*from Hanson *et al.* (1997) and **calculated from data presented by Fishelson (1997).

Parameter name	Description	Value
Consumption Eq. 2		
CA	Intercept for a 1g fish at <i>CTO</i>	0.603
CB	Coefficient of mass dependence	-0.465
CQ	Q ₁₀ value for consumption	3.1
CTO	Optimum temperature (°C) for consumption	29.8
CTM	Maximum temperature(°C) for consumption	34.5
Respiration Eq. 2		
RA	Intercept for a 1g fish at <i>RTO</i> (g·g ⁻¹ ·d ⁻¹)	0.0085
RB	Slope of allometric respiration function	-0.28
RQ	Q ₁₀ value for respiration	2.08
RTO	Optimum temperature for respiration	32
RTM	Maximum temperature (°C) for respiration	34.5
ACT	Activity multiplier	1.8
SDA*	Specific Dynamic Action	0.2
Egestion/Excretion		
FA**	Proportion of food consumed egested	0.2
UA	Proportion of food consumed excreted	0.06
Energy Density		
Predator	KJ·g ⁻¹ of lionfish wet weight (w)	ED = 2.708w ^{1.504}
Prey	KJ·g ⁻¹ of <i>Fundulus heteroclitus</i>	4.58
Reproduction	Percent mass released per spawn (female)	1

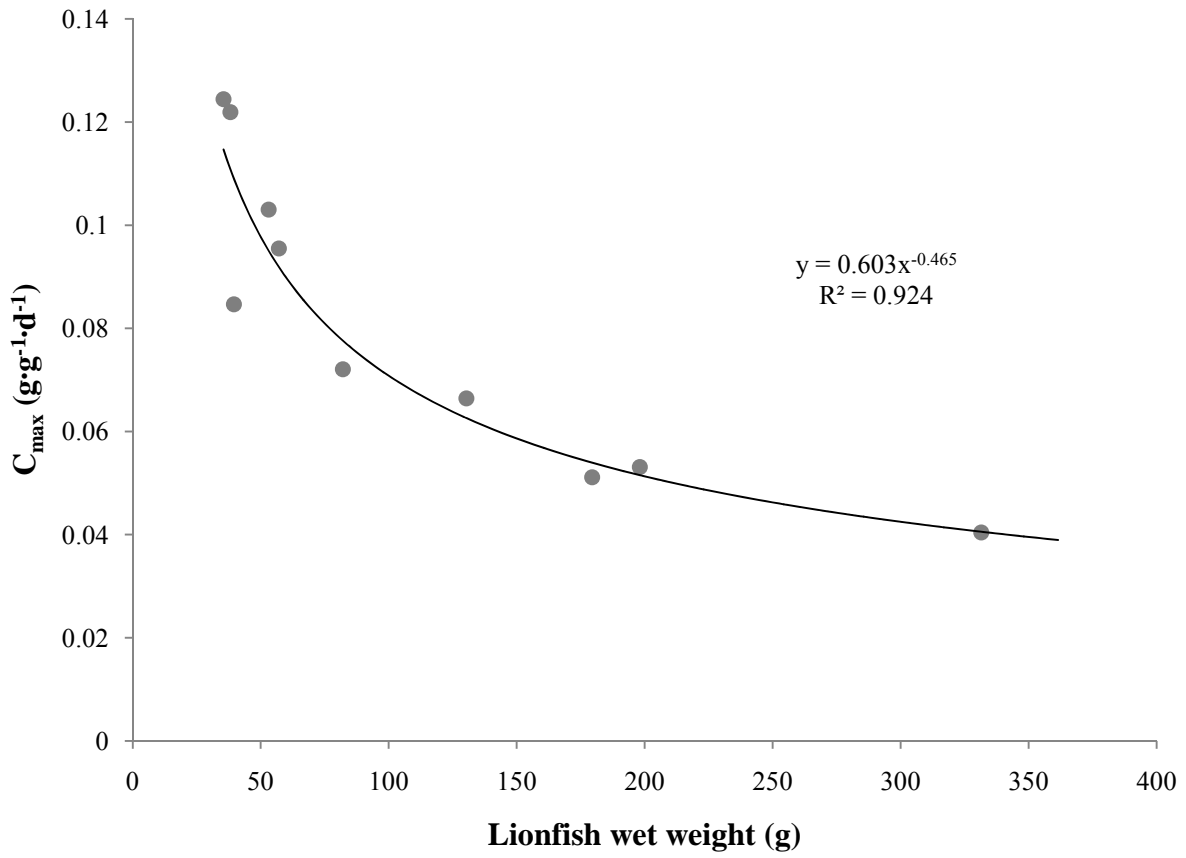


Figure 1. Weight-dependent mass-specific maximum consumption rates of lionfish from laboratory experiments at 29.7° C.

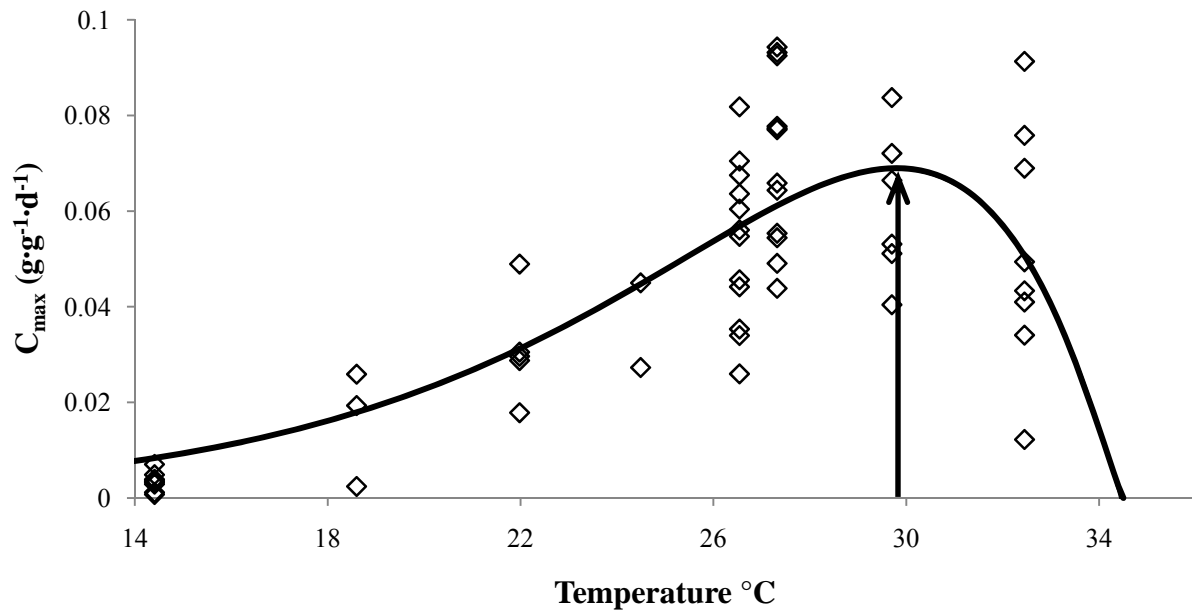


Figure 2. Weight-specific maximum consumption rate of lionfish 71-400g (N=52). The line represents $f(T)$, the temperature-dependence function for consumption. The arrow indicates the optimal temperature for consumption, $CTO = 29.8^{\circ} \text{C}$.

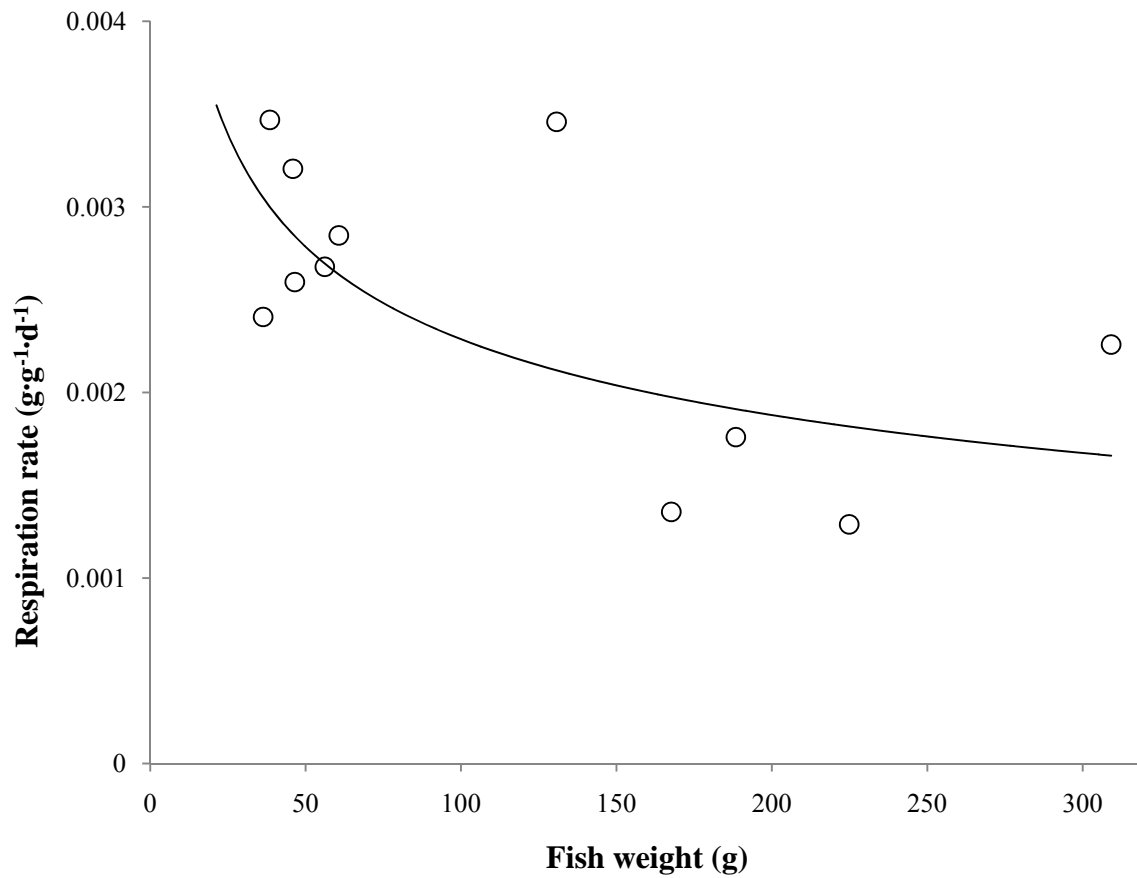


Figure 3. Weight-specific resting metabolic rate in $\text{g O}_2 \cdot \text{g of lionfish}^{-1} \cdot \text{d}^{-1}$ of lionfish 36-309 g. (N=11) at 29.1°C . *RA* (intercept) and *RB* (slope) for equation 2 in Fish Bioenergetics 3.0 generated from the least-squares regression equation (negative power function).

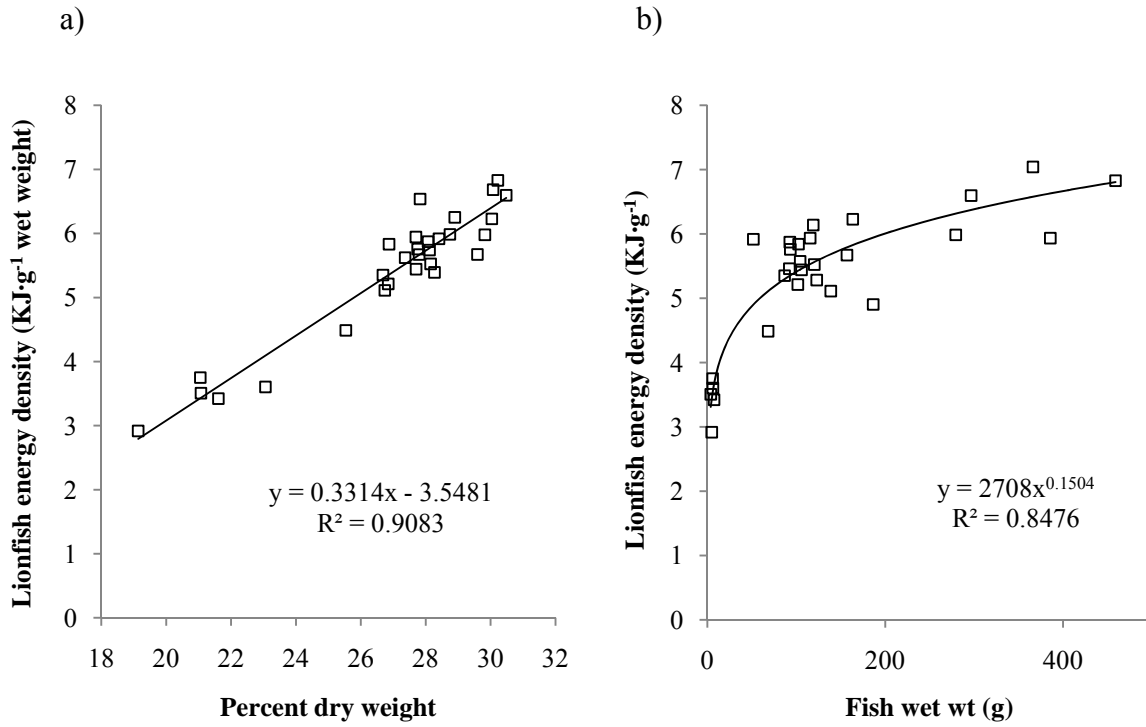


Figure 4. a) Lionfish energy density as a function of % dry weight, i.e. $100 \cdot (\text{dry weight/wet weight})$ (N=29). b) Relationship between fish wet weight and energy density (N=29).

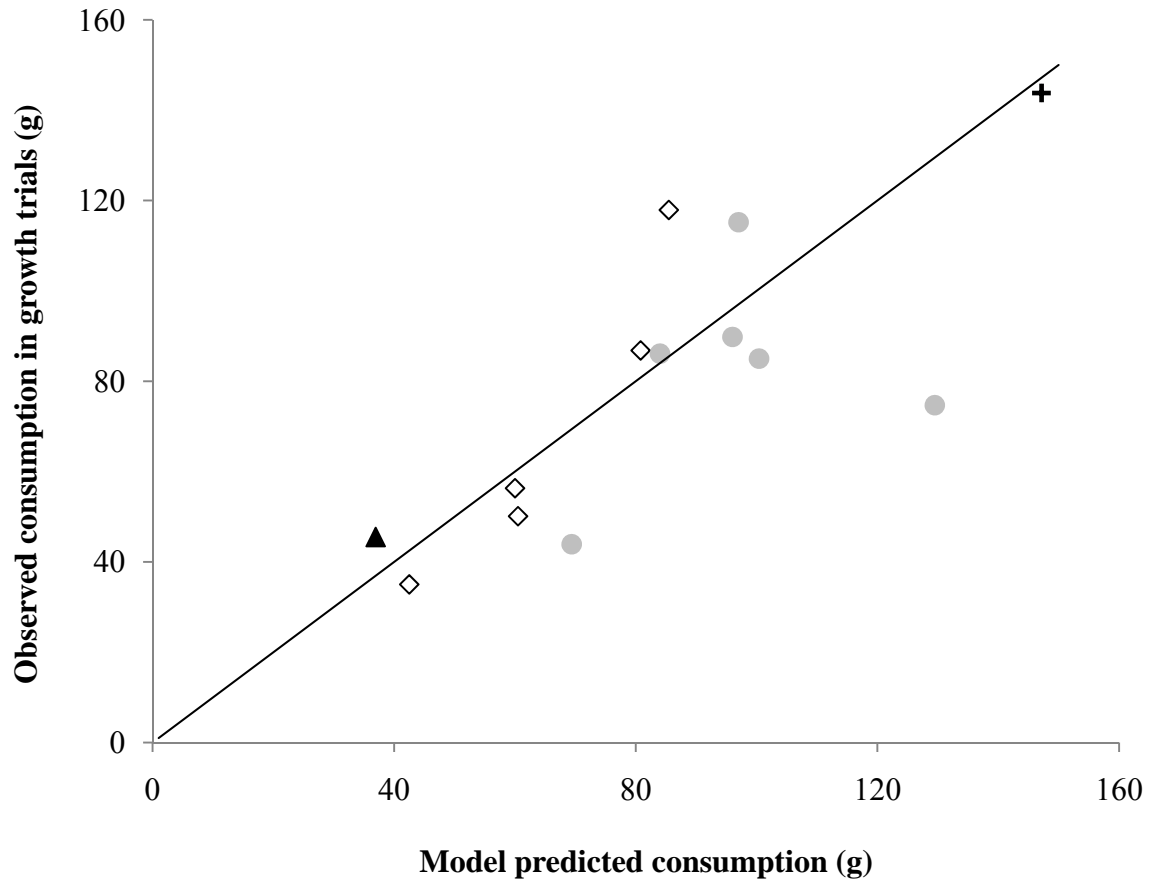


Figure 5. Model performance. Each point represents the model-predicted consumption based on observed growth and the actual consumption observed during 19-20 d laboratory trials. The line signifies a 1:1 relationship. Four categories of mean water temperatures are represented
 ▲ = 18° C, ◇ = 21-22° C, ● = 24-26° C, and + = 28° C.

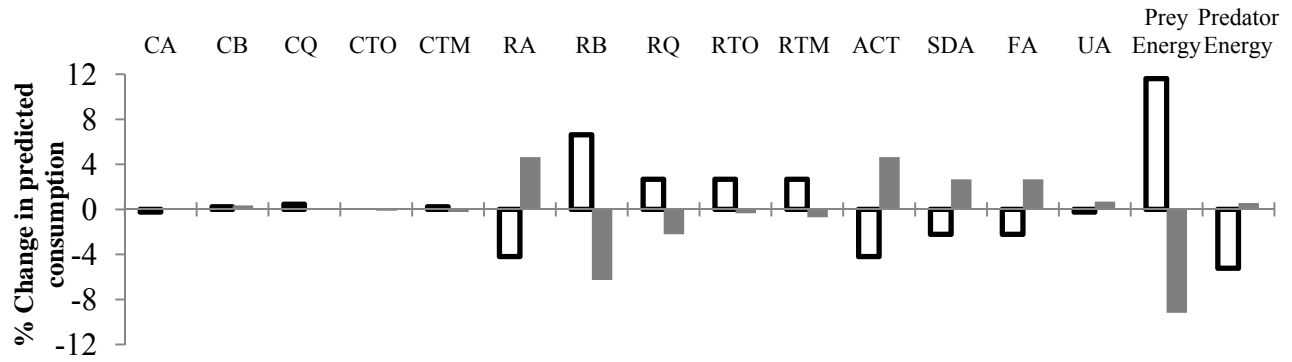


Figure 6. Sensitivity analysis. Bars indicate the percent change in predicted consumption of a lionfish that grew from 146.6 g to 168.7 g during a 19 d growth trial with a mean temperature of 24.8° C. One parameter was altered for each model run. The grey bars indicate the parameter values increased by 10% and the white bars indicate the parameter values reduced by 10%. The original model prediction was 86.1 g (0%) and the measured consumption was 84.5 g of *Fundulus heteroclitus*.

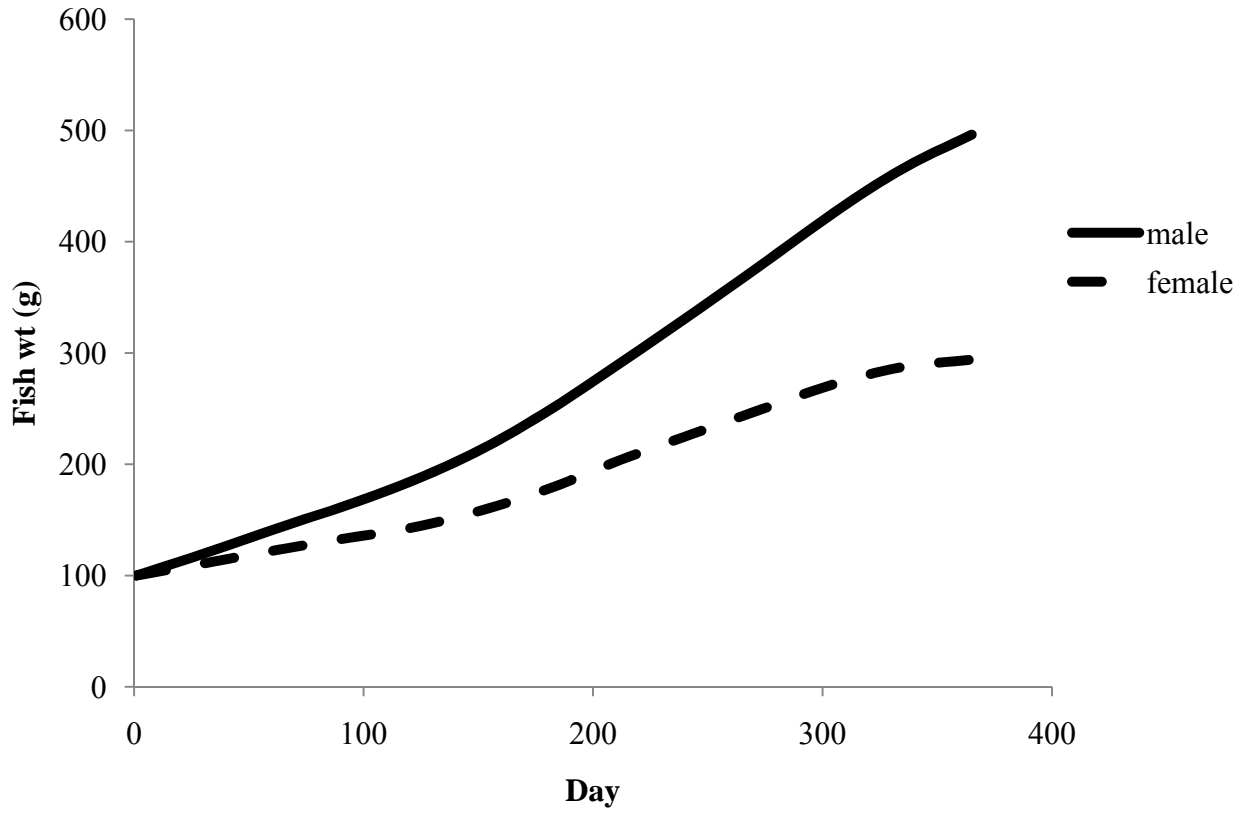


Figure 7. Model simulation depicting differences in sex-specific growth of lionfish due to female spawning every 4 d over the course of one year. Initial start weight is 100 g and consumption rate is $p = 0.6$.

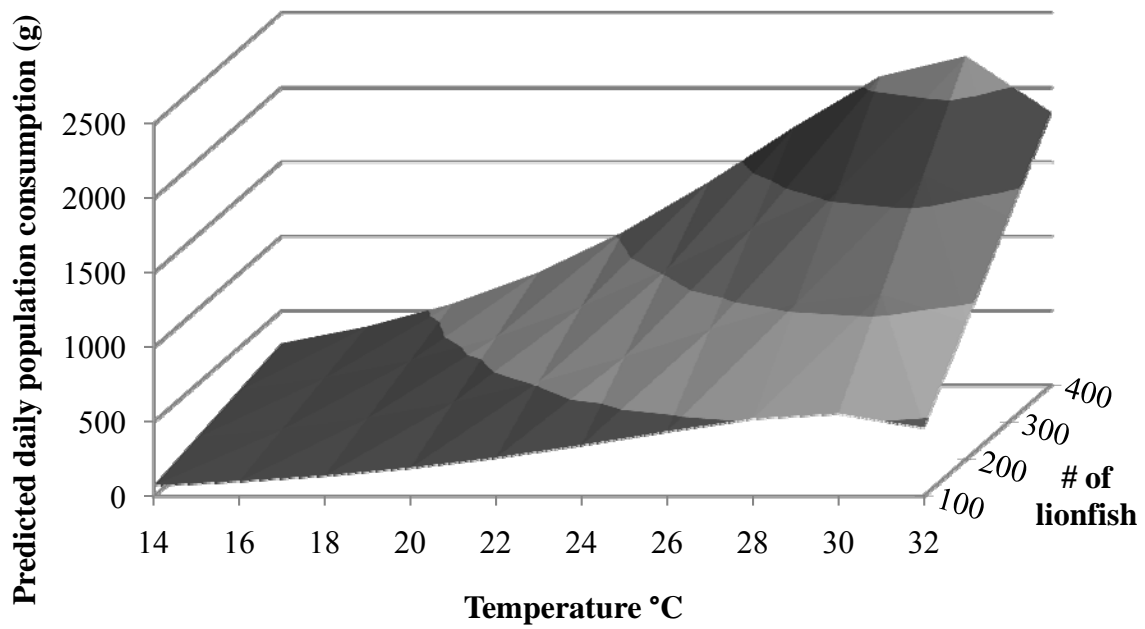


Figure 8. Effect of lionfish density and temperature on daily consumption rate of lionfish populations feeding at $p = 0.6$.

CHAPTER 3: CONCLUSION

Future Model Applications

The bioenergetics model presented in Chapter 2 is a valuable tool for quantifying the consumptive impacts of lionfish. Understanding lionfish physiology and metabolic characteristics provides a fundamental framework for assessing the ecological ramifications of this unprecedented invasive species. While a basic model application was demonstrated, more complex scenarios can be executed at broader and finer spatial and temporal scales.

Perhaps the most useful application of the model is the ability to model prey consumption across the habitats where lionfish are capable of surviving, as the model encompasses the range of thermal tolerance. The availability of high quality site-specific data will improve model predictions. Currently, there are several reports on reef fish assemblages in the Caribbean (e.g. Lang, 2003) using a variety of visual survey methods and habitat classifications. The reef fish survey data collected in recent decades will serve as a reference to measure how lionfish change fish communities. Linking fish community data, recruitment rate estimation, and lionfish metrics for the same site will provide the opportunity to test and calibrate the model in the field and begin to understand the role of invasive lionfish in the trophodynamics of reef communities. The following data are required to simulate daily lionfish population consumption for a specific site or region: 1) estimates of lionfish densities, 2) size-structure of lionfish populations, 3) sex ratio of populations, 4) lionfish growth rates, 5) water temperature, 6) diet composition, and 7) energy density of diet constituents. To model populations over longer time scales, it is important to estimate annual and seasonal mortality, recruitment and spawning frequency.

Trophic Impacts of Invasive Lionfish

Once accurate predictions of lionfish consumption are obtained, a more difficult task is interpreting how this consumption will affect community structure. Multiple factors have been indicated as affecting the community structures of reef fishes such as latitude (Ferreira *et al.*, 2004; Floeter *et al.*, 2004), habitat complexity (Ferreira *et al.*, 2001), fishing pressure (Roberts, 1995; Jennings & Polunin, 1996), variable recruitment rates (Tolmieri *et al.*, 1998), presence of adjacent nursery habitats (Mumby *et al.*, 2004), changes in invertebrate community (Lessios *et al.*, 1984; Robertson, 1991), presence of abundant non-native species (Dierking, 2007), and island biogeography (Sandin *et al.*, 2008). Coral reefs are the native habitats for lionfish; however, in the invaded range they are also found in or on rocky hardbottoms, blue holes, canals, artificial reefs, shipwrecks, and mangroves (Morris & Akins, 2009). Since reported lionfish densities have been highest in coral reef habitats, and coral reefs are prevalent in warm climates where lionfish consumption rates are highest, the consumptive effects on reef fish communities are of specific concern. Coral reef communities are complex, and subject to a variety of environmental and anthropogenic stressors. As demonstrated in Chapter 2, invasive lionfish is another potential stressor for coral reefs of the Northwestern Atlantic, Caribbean Sea, and Gulf of Mexico.

Marine reserves are becoming an increasingly popular management tool, especially in coral reef habitats. Marine reserves are geographic areas that are protected from extractive and destructive activities that are intended to conserve resources within that area and enhance the resources in surrounding areas (Lubchenco *et al.*, 2003). Many studies have demonstrated that fish community structure within no-take reserves is significantly different from unprotected areas (Côté *et al.*, 2001). Larger predatory fish within reserves (Polunin & Roberts, 1993) are

attributed to the absence of human fishing pressure within the reserve (Roberts, 1995; Mosqueira *et al.*, 2000). Remote areas that contain “pristine” reef environments that have not been heavily fished typically have a high biomass of large predators and lower biomass (by %) of small forage fish (Friedlander & DeMartini, 2002).

Several studies have focused on the presence, abundance, and community effects of large piscivores on global (e.g. Jackson *et al.*, 2001; Myers & Worm, 2003), regional (Paddack *et al.*, 2009) and local (Roberts, 1995) scales. These observations may indicate that coral reef communities can support a large biomass of piscivores, and are out of balance. Areas that have a high biomass of prey may appear less susceptible to negative impacts from lionfish predation; however, this might represent greater niche availability (Morris & Whitfield, 2009). For example, Harbone *et al.* (2008) observed $2.2 \text{ kg} \cdot \text{ha}^{-1}$ of large serranids (groupers) and lutjanids (snappers) at sites outside the Exuma Cays Land and Sea Park (ECLSP) reserve, and $99 \text{ kg} \cdot \text{ha}^{-1}$ of these same species in similar habitats within the reserve. For perspective, the lionfish population modeled in Chapter 2, estimated to be a realistic 2008 Bahamian population, has a biomass density of $100 \text{ kg} \cdot \text{ha}^{-1}$. The ECLSP was established in 1958 (Chiappone & Sullivan-Sealey, 2000), so reef fish populations within the reserve are likely close to equilibrium. If lionfish populations are reaching biomass densities similar to unfished populations of predatory fishes that are absent due to fishing pressure, this supports the theory that lionfish may be exhibiting niche takeover of economically important exploited reef fish species with similar feeding habits. This theory is supported by Byers (2002) who postulated that invasive species in systems disturbed by anthropogenic stressors, including removal of apex predators by overfishing, may have a competitive advantage over the native populations.

Reef fish biomass density estimates typically are derived from visual census surveys with observed fish recorded in categories of estimated length, and then converted to biomass by the species-specific length-weight relationships available from Fishbase (Froese & Pauly, 2010) for most species. Three common visual census survey methods include the stationary point count method (Bohnsack & Bannerot, 1986), belt transect, and roving diver. Each method has unique biases, and all methods incorporate individual observer bias and human error. These different methods have been shown to be comparable (Bortone *et al.*, 1989; Watson & Quinn, 1997; Samoilys & Carlos, 2000), but differences have been demonstrated as well (Colvocoresses & Acosta, 2007). Using identical survey methods at sites before and after invasion and at different stages of the lionfish invasion will reveal what changes in fish community structure have occurred.

Analyzing census data to estimate prey availability and competitive interactions will be complimented by better estimates of lionfish growth rates in different habitats and temperature regimes. Field growth measurements will be valuable for calibrating the model to accurately reflect lionfish consumption rates within a community in the invaded range. Diet composition should be monitored within habitats, and estimates of the energy density of small coral reef fish common in lionfish diets should be obtained. These estimates are not available in the literature, but can have a significant impact on the model's estimation of consumption or growth (Chapter 2).

Additional measures of reef fish abundance and population dynamics will improve the predictions of lionfish impacts on these systems. For example, fishes from the family Gobiidae rank in the top three in importance in lionfish diets (Morris & Akins, 2009), but are generally excluded from visual census surveys due to small body sizes and cryptic nature. Currently,

research effort is being devoted to better estimating the composition and density of small forage fish communities that lionfish prey upon off the coast of North Carolina (R. Muñoz, National Marine Fisheries Service, personal communication) and the Bahamas (S. Green, Simon Frasier University, personal communication). Additionally, more habitat-scale rates of fish recruitment to reefs are needed. The findings of Albins & Hixon (2008) that lionfish consumption contributed to a 79% reduction in recruitment of settling juvenile fishes to experimentally manipulated patch reefs is striking; however, it is difficult to extrapolate that observation to larger reef systems.

Future Research Needs

Much has been learned about lionfish prior to and since the invasion was first documented, and much can be learned from the invasion. Some studies have reported on lionfish biology and ecology in the native range including dietary and feeding observations (Fishelson, 1975 and 1997; Harmelin-Vivien & Bouchon, 1976), reproductive behavior and physiology (Fishelson, 1975 and 1978) and venom structure description (Halstead, 1955). Morris *et al.* (2009) and Morris & Whitfield (2009) provide comprehensive reviews of the biology and ecology of lionfish in the invaded range and describe the invasion chronology, taxonomy, local abundance, reproduction, early life history and dispersal, venomology, feeding ecology, parasitology, potential impacts, and control and management options. Larval fish dispersal patterns in the Southeast U.S., Caribbean, and Gulf of Mexico can be investigated by tracking the spread of lionfish through the region and assertions regarding connectivity between regions and habitats can also be tested by studying the invasion chronology (Morris, 2009; Ahrenholz & Morris, in press).

The long term consequences of the lionfish invasion are difficult to predict. The rapid increase in population size will likely asymptote and the population will eventually reach density-dependent equilibrium. Site-specific variability will make identifying the carrying capacity of lionfish for each reef system complex. Continuous monitoring of lionfish populations in regions that have established populations e.g. North Carolina, Bermuda, (Whitfield *et al.*, 2002), and the Bahamas (Schofield, 2009), in addition to areas with recent documented sightings such as the Southern Caribbean (e.g. Aruba, Costa Rica, Panama, and Honduras) (USGS NAS, 2010) and the Southern Gulf of Mexico near the Yucatan Peninsula (Aguilar-Perera & Tuz-Sulub, 2010) is needed to quantify population growth, and predict and possibly mitigate the impacts.

Human fishing pressure has attributed to the collapse of hundreds of species of marine organisms worldwide (Jackson *et al.*, 2001), and may be the only way to control lionfish populations (Morris & Whitfield, 2009). Effective capture methods for lionfish include net and spearfishing by divers; some traps have been effective while limited captures have been reported with hook and line (Morris & Whitfield, 2009). It is important to consider the protection of native fish stocks such as snapper-grouper species currently undergoing stock rebuilding, so promotion of harvest methods that target lionfish with little or no incidental harvest or bycatch is prudent. Lionfish are considered excellent table fare and small individuals have a high value as aquarium specimens; thus, economic incentive for the harvest of lionfish by humans does exist. Current management options and control strategies being considered in the U.S. include developing federal, regional or state fishery management plans, as well as control and monitoring within National Parks, National Marine Sanctuaries and Marine Protected Areas (MPAs) (Morris & Whitfield, 2009).

The results of this research indicate that lionfish are likely to impact reef fish communities in the Western Atlantic and Caribbean, and that a quantitative energetic model based on laboratory parameters will aid in predicting and measuring those impacts. Future research should focus on the reef fish community and trophic structure, prey community replenishment rates, lionfish growth rates and feeding rates. Ecosystem network models exist for Caribbean coral reef systems (Opitz, 1993, Deehr *et al.*, 2007) and incorporating the lionfish energetics reported here should be investigated. The impact of invasive lionfish is likely to vary by location and less disturbed areas, such as marine reserves, may be less vulnerable than sites with few native apex predators. If this hypothesis is shown to be valid, it may strengthen the movement towards use of MPAs as a management tool.

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BIOGRAPHICAL SKETCH

My childhood in Old Saybrook, Connecticut was spent playing in the tidal pools and sand flats of Long Island Sound. Once I outgrew building hermit crab cities and catching green crabs off the dock, I hit the waterways in search of big striped bass. This passion remained with me after high school, and I received a bachelor's degree in Marine Biology from the University of Maryland in 1999. The first step on my career path was conducting state Fisheries Independent Monitoring in Charlotte Harbor, Florida. My grandfather's sister, whom I barely knew, lived in Port Charlotte; and my 88 year-old Aunt Rose gave me a place to live, taught me to cook, and became my best friend. I found another lifelong friend that year as I fell in love with my beautiful wife Shayna, who lived down the street, and we have been together ever since. Next, I spent two years working for the National Marine Fisheries Service in Sandy Hook, New Jersey trawling the mighty Hudson River and conducting larval fish experiments. For four years I worked with the Sustainable Marine Aquaculture program of the U.S. Department of Agriculture at Harbor Branch Oceanographic Institution in Fort Pierce, Florida culturing Florida pompano. A leap of faith has brought me to North Carolina, where I have been fortunate to gain employment doing all the things I love: boating, fishing, diving, and growing fish as a contract employee with the National Ocean Service in Beaufort. My greatest joy of all arrived this past year, my wonderful son Noah, whose laughter brightens my soul every single day.

APPENDIX



Animal Care and Use Committee
East Carolina University
212 Ed Warren Life Sciences Building
Greenville, NC 27834
252-744-2436 office • 252-744-2355 fax

July 13, 2009

Anthony Overton, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Overton:

Your Animal Use Protocol entitled, "Bioenergetics Model for Assessing Trophic Impacts of Lionfish, *Pterois Volitans/Miles*," (AUP #D232) was reviewed by this institution's Animal Care and Use Committee on 7/13/09. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

A handwritten signature in cursive script that reads "Robert G. Carroll, Ph.D."

Robert G. Carroll, Ph.D.
Chairman, Animal Care and Use Committee

RGC/jd

enclosure